REMARKS

Reconsideration of this application is respectfully requested.

Applicants acknowledge the allowability **claims 1-7, 20-31 and 41-49** as indicated in the Office Action.

Amendments to the Claims

New claims 50-56 are added. Claims 50, 52 and 53 are supported by original claim 8 and by the specification at, for example, page 15, lines 29-31, page 16, lines 15-18 and page 17, lines 15-19. Claim 51 is further supported by original claim 9. Claim 54 is supported by the specification at, for example, page 16, lines 9-14, 19 and 27-29. Claim 55 is supported by the specification at, for example, page 16, lines 19-26. Claim 56 is supported by original claim 12.

The Examiner is thanked for pointing out the discrepancy in claim 13. Applicants have amended claim 13 for clarity. Although this amendment is made in response to a rejection under 35 U.S.C. § 112, Applicants submit that the amendment is only for clarification of what would be readily apparent to persons in the art reading the disclosure, and that the amendments should not further limit the claims.

Rejections under 35 U.S.C. § 112

The August 6, 2004 Office Action rejects claims 8-12 under 35 U.S.C. § 112, second paragraph as being ambiguous. In particular, the Office Action states that it is unclear what modulators are referred to in the phrase "modulators of cellular accumulation mechanisms" and "modulator" in claim 8; and what "efflux mechanism" and "influx mechanism" are being referred to in claims 10-11. Applicants traverse and request reconsideration.

First, applicants note that the term "modulator," as recited in claim 8, line 4 and claim 9, lines 2 and 3, refers to the "modulators of cellular accumulation mechanisms," recited in claim 8, lines 1-2. Accordingly, this particular rejection should be withdrawn.

Second, as explained in the specification at, for example, page 15, lines 12-20 and page 15, line 28 through page 17, line 24, cellular accumulation mechanisms include various transport systems within a cell that affect the accumulation of anti-tumor drugs (or other compounds) by altering the ability of the compound to move into and out of a tumor cell. Several such cellular

accumulation mechanisms are known in the art. By way of background, an excerpt from "The Encyclopedia of Cancer" describing one such efflux pump is attached. By altering drug accumulation, these mechanisms can adversely affect the effectiveness of a drug in treating a tumor. Additionally, it is known that it is desirable to maximize drug accumulation in tumor cells while minimizing drug accumulation, and hence toxicity, in normal host cells.

Once a mechanism of resistance is determined for a tumor, the search begins for a way to reverse the resistance, or "modulate" it. Thus, the discovery of the efflux pump MDR triggered a large number of preclinical and clinical studies to find agents that would reverse or overcome the resistance. A "modulator" or "modulator of cellular accumulation mechanisms" is a chemical entity that modulates; i.e. changes, the expression or activity of adverse cellular systems, such as efflux or influx pumps, in tumor cells, or drug intake in healthy cells. If properly selected, a modulator enhances drug concentration in tumor cells or changes or reduces baseline drug concentration in normal host cells. It is known that modulators can be co-administered with anti-tumor drugs, and this is not infrequent in anti-cancer therapies.

One of the insights of this invention is that it is the balance between influx and efflux that determines the net accumulation of drug within a tumor, and it is the net accumulation that is a determinant of response or resistance. Thus, while it might be useful to separately determine influx or efflux, the critical measurement would be net accumulation, which is elegantly probed by the radiolabeled version of the drug of interest. Therefore, the invention does not measure the activity of a specific modulator, efflux mechanism or influx mechanism, but rather measures the result, cellular accumulation, which is the determinative factor for evaluating a particular therapy regimen.

As recited in claims 8-12 and new claims 50-56, the present invention includes a method for measuring the effectiveness of modulators and, therefore, potentially improving the efficacy of an anti-tumor drug. As described in the specification, by monitoring modulator effectiveness through measuring drug accumulation in a tumor or host cesll, suitable modulators can be selected. Persons skilled in the art would understand this and understand what is meant by "cellular accumulation mechanisms," "modulators," and "modulators of cellular accumulation mechanisms." Because these systems are known, claim 8, and claims dependent therefrom, are clear and unambiguous to persons skilled the art and the rejections should be withdrawn. (See

MPEP § 2173.02 ("In reviewing a claim for compliance with 35 U.S.C. 112, second paragraph, the examiner must consider the claim as a whole to determine whether the claim apprises one of ordinary skill in the art of its scope and, therefore, serves the notice function required by 35 U.S.C. 112, second paragraph . . ."(emphasis added); *Morton International Inc. v. Cardinal Chemical Co.*, 5 F.3d 1464, 28 USPQ2d 1190 (Fed. Cir. 1993) ("Whether a claim is invalid for indefiniteness requires a determination whether those skilled in the art would understand what is claimed when the claim is read in light of the specification.")).

Third, as mentioned above and described in the specification, various systems that affect drug accumulation are known in the art. Such systems include, for example, efflux pumps or transporters, which move drugs and other compounds out of a cell, and influx pumps or transporters, which move drugs and other compounds into the cell. These meaning of these terms would be well understood by persons of ordinary skill in the art. No specific efflux pumps or influx pumps are required by the invention; persons of ordinary skill in the art would know which mechanisms may be acting and which modulators may be useful in modulating activity of those mechanisms. Accordingly, claims 10 and 11 are clear and unambiguous to persons of ordinary skill in the art, and the rejections should be withdrawn.

Finally, new claims 50-56 are set forth alternate language to define the method and more specifically recite MDR modulators. Applicants submit that these claims are similarly allowable.

CONCLUSION

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. Accordingly, Applicants request that the Examiner indicate the allowability of claims 1-31 and 41-56 and the application pass to issue. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is hereby invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment is respectfully requested.

Respectfully submitted,

Date: December 6, 2004

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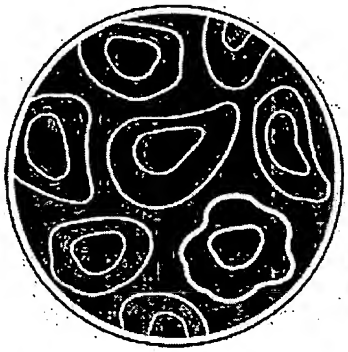

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Multidrug Resistance I: P-Glycoprotein

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I. Role of the Multidrug Efflux Pump, P-glycoprotein, in Multidrug Resistance in Cancer Cells
II. Structure and Mechanism of Action of P-glycoprotein
III. Normal Cellular Function of P-glycoprotein
IV. Role of P-glycoprotein in Drug Pharmacokinetics
V. Implications of Studies on P-glycoprotein for Treatment of Cancer

GLOSSARY

apoptosis A process of programmed cell death stimulated by natural factors or anticancer drugs.

ATP-binding cassette A protein sequence found in a related family of proteins, which allows the cellular source of energy, ATP, to bind to these proteins and be utilized for various transport processes.

extracellular space The space between cells in a tissue, or outside of a cell growing in tissue culture, from which drugs are taken up by a cell.

hydrophobic Of or relating to a property of molecules that prevents them from dissolving in water and enables their solution in cell membranes.

induction A form of regulation of gene expression in which an environmental alteration (frequently the presence of a specific chemical) leads to the expression of a gene. In the case of drug resistance, the cytotoxic compound may turn on expression of a protective detoxifying mechanism. Induction is contrasted with mutations that are permanent, inherited changes in genes, which may result in drug resistance.

lipid bilayer The collection of lipids that makes up the plasma (outer) membrane of cells. Phospholipids are arranged in two layers so that their polar water-soluble heads are exposed to either the inner (cytoplasmic) or the outer (extracellular) surface.

multidrug resistance The development of cross-resistance to many different drugs as the result of a single biochemical change or a single genetic alteration. In its strictest sense, cross-resistance should refer to resistance to drugs with different structural features and different cytotoxic targets.

multifactorial multidrug resistance Resistance resulting from a change in many different resistance mechanisms, not a single mechanism, as is the case in classical multidrug resistance.

natural product A pharmacological agent that comes from the natural environment and is not synthesized *de novo* in the laboratory.

P-glycoprotein An energy-dependent plasma membrane pump responsible for multidrug resistance to many natural product drugs and their derivatives. In the human, P-glycoprotein is the 1280 amino acid product of the *MDR1* gene.

pharmacogenomics A new term coined to indicate the study of those inherited changes in proteins and in the factors that regulate their expression, which account for inherited differences in metabolism, uptake, distribution, and excretion of drugs.

photoaffinity labeling The use of chemical compounds that can be activated by light so as to form chemical linkages with other macromolecules to which they are bound. When such photoaffinity labels are radioactive, they can be used to identify such binding macromolecules.

plasma membrane The lipid bilayer and associated proteins and other molecules that make up the outer surface of cells.

substrate A molecule that is acted upon by a macromolecule so as to change its chemical composition or to transport it within, into, across, or out of cells.

transgenic mice Mice whose genes have been altered in the laboratory to enable the study of the function of a specific gene or genes. Transgenic mice may carry new genes, or have altered genes, including alterations that totally knock out the expression of specific genes.

transmembrane domains These parts of proteins inserted into the lipid bilayer of cell membranes.

xenobiotics Chemical materials, usually natural products, that are toxic to cells. Some of these are present in food and microorganisms and are ingested inadvertently, whereas others are used in the treatment of cancer and other diseases.

Multidrug resistance is the phenomenon by which cancer cells display simultaneous resistance to many different anticancer drugs that are chemically dissimilar and that do not have the same cytotoxic target within the cancer cell. It is now known that multidrug resistance can have many different causes, including failure of cancer cells to accumulate drugs, to metabolize them to toxic products, and to activate cell death pathways, but for many years this phenomenon of broad drug resistance was mysterious to investigators who studied resistance to anticancer drugs.

I. ROLE OF THE MULTIDRUG EFFLUX PUMP, P-GLYCOPROTEIN, IN MULTIDRUG RESISTANCE IN CANCER CELLS

The first big breakthrough in this field was made in 1985 when three groups published the sequence of an energy-dependent plasma membrane transporter, or P-glycoprotein, the product of the *MDR1* gene. P-glycoprotein could detect many different lipid-soluble anticancer drugs and pump them out of the cell so as to prevent accumulation to toxic levels (see Fig. 1). The relative lack of specificity of this transport pump, and the fact that many anticancer drugs are lipid-soluble natural products, accounted for its ability to confer multidrug resistance on cancer cells. Table I summarizes some of the known substrates for the P-glycoprotein pump, which include not only anticancer drugs, but also many other important pharmacologic agents in common use in the clinic. Subsequently, other energy-dependent drug efflux pumps were discovered, including the *MRP* family of transporters.

Once antibodies and molecular probes were available for detection of P-glycoprotein in cancer cells, it was possible to ask whether cancers from patients, with or without exposure to anticancer drugs, expressed this efflux pump. It was known by the late 1980s that many different kinds of cancers express

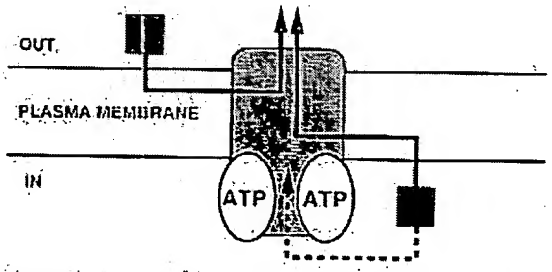

FIGURE 1 Diagram illustrating the potential mechanism of action of P-glycoprotein as it removes drugs (solid squares) from the lipid bilayer, either as they are entering the cell or once they have accumulated intracellularly. Solid lines represent likely pathways of drugs through the transporter. The dotted line is another possible pathway in which drugs are pumped directly out of the cytoplasm. [From Gottesman, M. M., and Pastan, I. (1996), "Drug resistance: alterations in drug uptake or extrusion." In "Encyclopedia of Cancer," Vol. 1, A–F (J. R. Bertino, Ed.), p. 555. Academic Press, San Diego, CA.]

TABLE I
Selected Substrates of P-glycoprotein

Anticancer drugs
Vinca alkaloids (vincristine, vinblastine)
Anthracyclines (doxorubicin, daunorubicin, epirubicin)
Epipodophyllotoxins (etoposide, teniposide)
Paclitaxel (taxol)
Actinomycin D
Topotecan
Mithramycin
Mitomycin C
Other cytotoxic agents
Colchicine
Emetine
Ethidium bromide
Puromycin
Cyclic and linear peptides
Gramicidin D
Valinomycin
N-Acetyl-leucyl-leucyl-norleucine
Yeast a-factor pheromone
HIV protease inhibitors
Ritonavir
Indinavir
Saquinavir
Other compounds
Hoechst 33342
Rhodamine 123
Calcein-AM

P-glycoprotein at levels high enough to confer resistance to anticancer drugs and that the presence of P-glycoprotein frequently correlated with resistance to drugs known to be substrates for transport by P-glycoprotein. For example, epithelial cancers of the colon, small intestine, pancreas, adrenal, and kidney, derived from tissues that normally express P-glycoprotein, usually expressed high levels of this transporter. Although P-glycoprotein pump activity is not the only reason for the multidrug resistance of these epithelial tumors, expression of P-glycoprotein does appear to be a barrier to effective therapy in these cancers with many different standard anticancer drugs.

Some cancers, which did not express high levels of P-glycoprotein initially, began to express high levels after many cycles of selection in anticancer drugs. Examples include leukemias, lymphomas, myelomas, and ovarian cancer. In these cases, it is presumed that the toxic drug kills most of the sensitive cells in the original population, and only those cells that express higher levels of P-glycoprotein, as a result of a mutation in the cancer cell affecting regulation of expression of this pump, are able to survive and multiply. Evidence suggests that, to some extent, cytotoxic drugs can directly turn on the expression of P-glycoprotein and that this phenomenon might contribute to clinical multidrug resistance in some cases. However, in cultured cells, the survivors of drug selection appear to express P-glycoprotein in a stable manner, thereby arguing in favor of selection of a preexisting mutant cancer cell rather than an induction mechanism that would not result in stable, long-term expression of P-glycoprotein.

Finally, it appears that the malignant transformation process itself can result in turning on expression of the *MDR1* gene. Examples include some leukemias (especially the blast transformation of cells, which occurs in chronic myelogenous leukemia) and neuroblastomas in children. It is not known why this occurs, but presumably the malignantly transformed state itself results in induction of the P-glycoprotein gene, and the resulting tumor cells are multidrug resistant. Another possibility is that expression of P-glycoprotein enhances the malignant phenotype. There is preliminary evidence in some model systems that expression of P-glycoprotein has an antiapoptotic effect. These observations are consistent with the idea that P-glycoprotein may facilitate cell survival in tumors.

An important unanswered question is whether inhibiting the function of P-glycoprotein in tumor cells that express it will result in better responses to chemotherapy, i.e., is P-glycoprotein ever limiting in determining the response to chemotherapy? Despite many clinical trials that have addressed this issue, the answer is not yet available. Until there are potent, specific inhibitors of P-glycoprotein available, and these are tested in cancers known to express P-glycoprotein, we will not have this answer. Preliminary studies for some leukemias and myelomas suggest that improved response is possible when P-glycoprotein is inhibited, but the response in epithelial tumors seems minimal. Presumably this is because intrinsic multidrug resistance in epithelial cells is multifactorial, whereas resistance that occurs in cancers selected with anticancer

drugs is more likely to be due to a single cause. However, despite the fact that inhibiting P-glycoprotein in epithelial cancers does not sensitize these cancers to natural product anticancer drugs, it will be necessary in designing new drugs to keep in mind the presence of this drug efflux pump because drugs that do not accumulate in cells cannot kill them.

II. STRUCTURE AND MECHANISM OF ACTION OF P-GLYCOPROTEIN

The discovery of a pump that can recognize and extrude many different anticancer drugs led naturally to the question of how so many different substrates could be recognized by one protein and how the energy of ATP was harnessed to the pump process. The first step was to create a model of P-glycoprotein based on its known amino acid sequence. In the human, P-glycoprotein is encoded by 1280 amino acids. A total of 12 segments consist mostly of water-insoluble amino acids, which would be expected to cross the plasma membrane of the cell; 6 of these are in the amino-terminal half of the pump and 6 are in the carboxyl half. In addition, two regions are very similar to ATP-binding regions of other proteins, and in particular appear to have some sequence characteristics seen in a large family of proteins (approximately 50 are encoded in the human genome) involved in energy-dependent transport across cellular membranes. This family is known as the ATP-binding cassette (ABC) family of proteins. A hypothetical structure of P-glycoprotein based simply on the amino acid sequence and supported by data using specific antisera is shown in Fig. 2.

Based on photoaffinity-labeling studies using analogs of know substrates for P-glycoprotein and on mutational analyses in which individual amino acids in the transmembrane domains of P-glycoprotein are mutated,

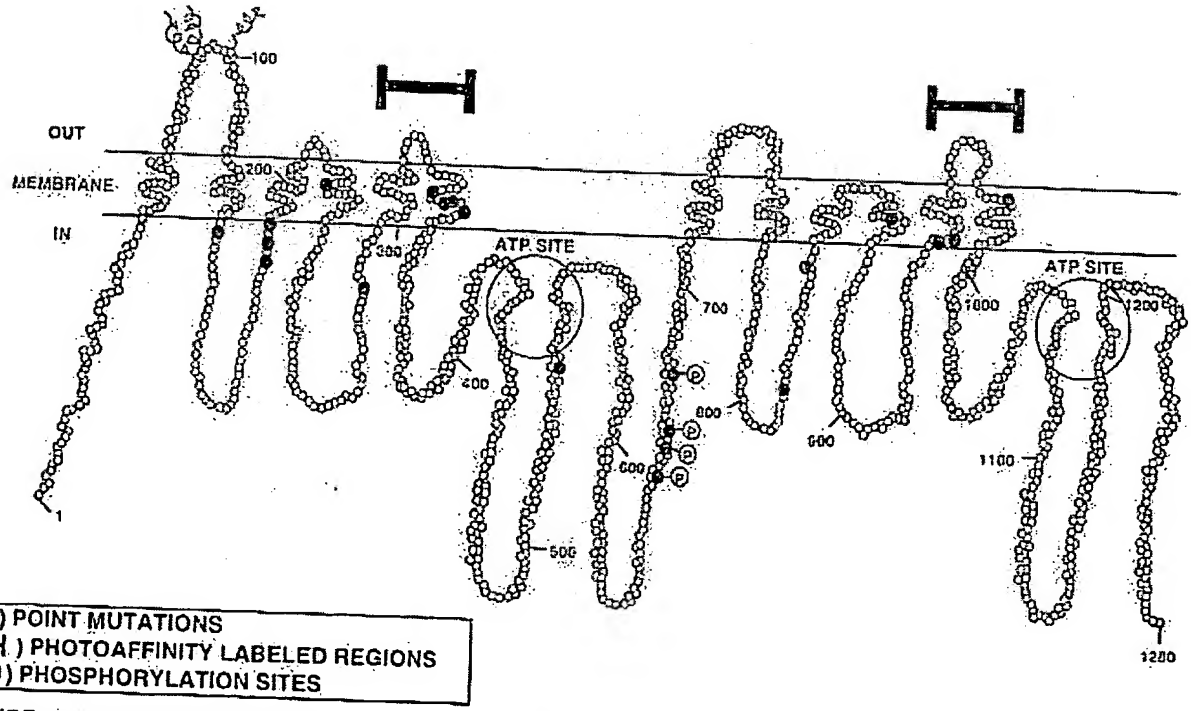

FIGURE 2 A hypothetical two-dimensional model of human P-glycoprotein based on hydropathy analysis of the amino acid sequence and its functional domains. In this schematic diagram, each circle represents an amino acid residue, with solid circles showing the positions of mutations that alter the substrate specificity of P-gp (for clarity, not all known mutations are shown). N-linked glycosylation sites are indicated by squiggly lines, and phosphorylation sites are shown as a circled P. Bars above the model show regions labeled with photoaffinity analogs. [Adapted from Gottesman, M. M., and Pastan, I. (1988). The multidrug transporter as a double-edged sword. *J. Biol. Chem.* 263, 12163.]

it appears that the major drug interaction sites on P-glycoprotein are in the transmembrane segments. This information, plus the knowledge that P-glycoprotein is able to decrease the rate of influx of drugs as well as increase their rate of efflux, and some studies showing a direct interaction of drugs dissolved in the plasma membrane with P-glycoprotein, has led to a model of P-glycoprotein as a "hydrophobic vacuum cleaner." In other words, P-glycoprotein detects and ejects drugs while they are still in the lipid bilayer (see Fig. 1). The specific drug interaction sites on P-glycoprotein appear to be quite complex and consist of a series of overlapping hydrophobic sites within the parts of the protein that transit the plasma membrane. This model explains the rather broad substrate specificity of P-glycoprotein, as the important interactions are hydrophobic and not ionic. The general finding that recognition of drugs depends more on hydrophobicity and size and shape rather than specific chemistry is also consistent with this hypothesis.

The presence of two ATP-binding/utilization sites on P-glycoprotein has also been puzzling. Data suggest that ABC cassette proteins require hydrolysis of two molecules of ATP to transport a single molecule of substrate, and data based on the transport of vinblastine by P-glycoprotein are consistent with this generalization. Why two molecules of ATP? Hydrolysis of one molecule of ATP appears to be sufficient to reduce the affinity of P-glycoprotein for its substrates, which probably corresponds to the change in shape that the pump undergoes as it extrudes drug from the lipid bilayer into the extracellular space. Very recent evidence points to the need for hydrolysis of a second molecule of ATP so that the pump can return to its initial high-affinity binding state and begin the pumping cycle all over again. Additional experimental evidence points to the need for two functional ATP sites (inactivation of one site results in loss of pump function), asymmetry of the two sites, and the alternating action of these two sites so that ATP hydrolysis cannot occur at both sites at the same time.

III. NORMAL CELLULAR FUNCTION OF P-GLYCOPROTEIN

The discovery of P-glycoprotein and its ability to recognize dozens, and perhaps hundreds of structurally dissimilar compounds, raised questions not only about its mechanism of action, but about how it had evolved and what its normal function might be. The finding that all living organisms so far studied, from bacteria to humans, have pumps similar to P-glycoprotein suggested that free living cells could not survive without a broad-spectrum pump for hydrophobic compounds. Whether this reflects the ubiquitous presence of toxic xenobiotics in the environment (most of which are themselves the products of life forms and may be part of the armaments of battle used as organisms strive to gain a selective advantage in their biological niches) or an intrinsic requirement for *MDR* genes to maintain cellular integrity is still not known with certainty. However, genetically engineered organisms such as transgenic mice lacking *mdr1* genes (in the mouse, there are two *mdr1* genes, called *mdr1a* and *mdr1b* or *mdr1* and *mdr3*), and other mammalian cells lacking *MDR1* expression, survive with normal life spans, suggesting that there is no absolute requirements for *MDR1* expression in cells. Moreover, mice without functioning *mdr1* genes are exquisitely sensitive to certain xenobiotics, but carry on other cellular functions with reasonable efficiency. Because many transport functions of P-glycoprotein in mammals are redundant with other ABC transporters, especially the *MRP* system, it is still possible that every living cell needs one or another member of this class of transporters to survive.

What sort of evidence exists suggesting cellular functions for P-glycoprotein other than to protect cells from toxic effects of xenobiotics? In general, overexpression of P-glycoprotein after gene transfer into various cell types or after selection in cytotoxic drugs has been used to study the effect of P-glycoprotein on cellular processes. Under these conditions, it is possible to show a reduction in sensitivity to apoptosis in lymphoid cells, resistance to certain enveloped viruses, including HIV, and altered plasma membrane lipid composition. Early studies of P-glycoprotein-expressing cells suggested a correlation with the metastatic potential of cancer cells. Use of inhibitors of P-glycoprotein results in altered cellular functions, but there are probably no totally specific P-glycoprotein inhibitors that can give definitive results about the function of P-glycoprotein alone. Use of ribozymes and antisense technologies to eliminate P-glycoprotein expression have so far not

totally eliminated the expression of P-glycoprotein, although some reductions in P-glycoprotein levels have been possible using this approach.

Once again, the use of mice lacking functioning *mdr1* genes has proved useful in determining the normal function of P-glycoprotein. As noted earlier, *mdr1* knockout mice live normal life spans and are sensitive to xenobiotics. More detailed studies of the function of individual tissues that normally express P-glycoprotein suggest subtle alterations in immune system function, not yet clearly defined, and relatively normal function of epithelial tissues of the gastrointestinal tract, liver, kidney, and pancreas.

IV. ROLE OF P-GLYCOPROTEIN IN DRUG PHARMACOKINETICS

The use of inhibitors of P-glycoprotein and the study of *mdr1* knockout mice have confirmed suspicions from the initial histochemical localization studies that *MDR1* played a major role in uptake, distribution, and excretion of toxic xenobiotics in mice and humans. Lack of expression of functional P-glycoprotein in the gastrointestinal tract dramatically enhances the absorption of several different drugs, including anticancer drugs such as taxol, the cardiac glycoside digoxin, and the antihistamine fexofenadine. This is manifested as much higher blood levels of these compounds after oral dosing. Furthermore, distribution of such drugs in the body is altered in the absence of function P-glycoprotein, manifested as increased brain levels of compounds such as the antihelminthic ivermectin, the anticancer drug vinblastine, and the antidiarrheal narcotic analog loperamide. This is attributed to the abrogation of the blood–brain barrier for these compounds due to the absence of P-glycoprotein in capillary endothelial cells in the brain. Similar effects of loss of P-glycoprotein at the placental barrier would be expected to result in increased teratogenicity of certain compounds, which are P-glycoprotein substrates, and perhaps toxic and/or mutagenic effects on germ cells in the testis and ovary.

Circulating cells, such as T cells and macrophages, which normally express P-glycoprotein, might become sensitized to drugs and xenobiotics if their P-glycoprotein levels are altered. Because the HIV protease inhibitors are P-glycoprotein substrates, this phenomenon has led to the suggestion that the cellular availability of drugs such as this could be manipulated by altering levels of functional P-glycoprotein. Conversely, resistance to certain drugs could occur at the cellular level because of variations in expression of P-glycoprotein that might be genetically determined (see later).

Finally, excretion by the liver (in bile) and kidney (in urine) of P-glycoprotein substrates is substantially reduced for many drugs in mice lacking P-glycoprotein. This results in a decreased rate of clearance for drugs and increased accumulation of drugs in the bloodstream and tissues. Thus, use of inhibitors of P-glycoprotein, or alterations in levels of functional P-glycoprotein that might be genetically determined, could have fairly profound effects on the blood and tissue levels of many different drugs by increasing absorption, decreasing excretion, and altering distribution into tissues protected by P-glycoprotein barriers or into cells expressing P-glycoprotein. These effects of P-glycoprotein are summarized in Fig. 3.

Evidence is beginning to be published suggesting that levels of P-glycoprotein in the gastrointestinal tract may vary considerably from individual to individual, perhaps accounting for differences in absorption of drugs that are primarily P-glycoprotein substrates. One study suggests genetic linkage with a single nucleotide polymorphism that does not change the sequence of P-glycoprotein, but is presumably linked to an alteration in a regulatory region that controls levels of P-glycoprotein in the gastrointestinal tract. Several different coding polymorphisms of P-glycoprotein have been described, but none to date have been shown to alter the ability of P-glycoprotein to pump drugs. Much more work is needed in this important area of pharmacogenomics.

V. IMPLICATIONS OF STUDIES ON P-GLYCOPROTEIN FOR TREATMENT OF CANCER

As we assemble a more complete picture of the biochemistry, pharmacology, and physiology of P-glycoprotein function, we can begin to make better guesses as to the role that manipulation of the *MDR1*

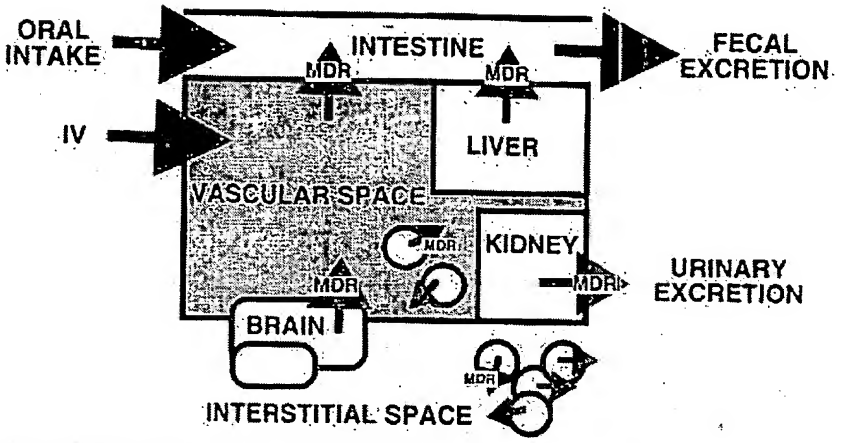

FIGURE 3 The effect of P-glycoprotein on pharmacokinetics and drug distribution is illustrated schematically. The multidrug transporter affects drug distribution throughout the body.

gene is likely to have in the future treatment of cancer. Some conclusions seem quite firm, whereas other are still speculative. First, it seems clear the P-glycoprotein is capable of conferring resistance to many anticancer drugs on cancer cells. Thus, as new drugs are developed, it will be necessary to determine whether cellular sensitivity to these drugs is affected by the presence of P-glycoprotein. If, as seems likely for the foreseeable future, we continue to be dependent for the treatment of cancer on the existing hydrophobic natural products, and new drugs that are also P-glycoprotein substrates, then we will have to learn to inhibit P-glycoprotein, and its many molecular analogs, as we try to cure and palliate cancer.

Second, despite earlier concerns, it seems likely that more potent, more specific inhibitors of P-glycoprotein, or inhibitors of related ABC transporters, can be developed and that their toxicity would be relatively limited and manageable. This conclusion comes from the studies with transgenic mice lacking P-glycoprotein, which have normal life spans under controlled laboratory conditions. These inhibitors will play a role not only in sensitizing P-glycoprotein-expressing cancers to anticancer drugs, but also in altering uptake, excretion, and cellular distribution of many different drugs. Using P-glycoprotein inhibitors it may be possible to give drugs orally that previously required intravenous administration or to deliver drugs to the brain that previously were only active outside of the central nervous system.

Third, it appears that alterations in the levels of expression of P-glycoprotein in different tissues, which may result from environmental exposures or from inherited alterations in pathways that regulate expression of the *MDR1* gene, or inherited polymorphisms within the coding region of the *MDR1* gene could account for variations among individuals in the way they respond to drugs. Such changes could have substantial effects on drug uptake, distribution, and excretion. It will become necessary in the future to catalog these variations in the *MDR1* gene and other genes, which affect metabolism, absorption, and excretion of drugs, in order to predict individual responses to different drugs.

Finally, cloning of the *MDR1* cDNA encoding P-glycoprotein has made it possible to transfer this gene into drug-sensitive cells, thereby conferring multidrug resistance on all recipient cells. Because the toxicity of anticancer drugs to sensitive tissues, such as epithelia and bone marrow, is a major dose-limiting problem in cancer chemotherapy, the ability to protect normal cells from this toxicity using transferred multidrug resistance genes has been the subject of much laboratory and clinical investigation. Although such gene transfer studies are limited by the low efficiency of existing vector systems and the safety of gene transfer of multidrug resistance genes is yet to be established, it is conceivable that multidrug resistance genes, such as *MDR1*, may prove useful in protecting normal tissues from cytotoxic effects of anticancer treatment.

See Also the Following Articles

EXTRACELLULAR MATRIX AND MATRIX RECEPTORS • MULTIDRUG RESISTANCE II: MRP AND RELATED PROTEINS • P-GLYCOPROTEIN AS A GENERAL ANTIAPOPTOTIC PROTEIN

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